Guidance for Industry

Immunotoxicology Evaluation of Investigational New Drugs

DRAFT GUIDANCE

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Immunotoxicology Evaluation of Investigational New Drugs

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I. INTRODUCTION

This guidance makes recommendations to sponsors of investigational new drugs (INDs) on (1) the parameters that should be routinely assessed in toxicology studies to determine effects of a drug on immune function, (2) when additional specific immunotoxicity studies should be conducted, and (3) when additional mechanistic information could help evaluate the significance of a given drug's effect on the immune system. This guidance is intended for drug products and does not apply to biological products.[2]

Five adverse event categories are discussed in this guidance.

1. Immunosuppression: Effects on the immune system that result in decreased immune function
2. Antigenicity: Specific immune reactions elicited by a drug and/or its metabolites
3. Hypersensitivity: Immunological sensitization due to a drug and/or its metabolites
4. Autoimmunity: Immune reactions to self-antigens
5. Adverse immunostimulation: Non-antigen specific activation of the immune system

[1] This guidance has been prepared by the Office of Review Management in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA).

[2] Sponsors of biological products should refer to the International Conference on Harmonisation (ICH) guidance *S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharamaceuticals* (July 1997).

II. BACKGROUND

A functional immune system is vital to human survival. Assessment of adverse effects on the immune system is therefore important in the overall nonclinical evaluation of drug toxicity. Evidence of immunotoxicity can usually be observed in standard nonclinical toxicology studies, but in some cases additional studies are important. Observation of immune system effects may also suggest that more specific follow-up studies should be considered.

III. EVALUATING IMMUNOTOXICITY MARKERS

Signs of immunotoxicity in nonclinical studies should be evaluated to determine whether more specific studies would be useful. When considering follow-up studies based on changes in clinical pathology parameters, observed signs should represent biologically significant effects. Changes in some parameters might not be cause for concern when the changes are small but statistically significant. For example, any decrease of more than 40 percent in total lymphocytes (Hannet et al., 1992; Luster et al., 1993) or 75 percent in granulocyte counts (Johansen 1983) could be significant, while changes less than 40 percent and 75 percent may be only suggestive of the immunotoxicity. Evidence of immunotoxicity in more than one species would cause more concern, even if large effects are not observed. Dose-related changes would also have additional significance, although threshold effects and *bell-shaped curve* phenomena, more commonly seen with biological agents, should be considered when evaluating whether to conduct additional testing.

Potential immunotoxic effects should be evaluated in terms of both dose and, when data are available, systemic exposure. Where possible, dose comparisons to clinical use should be based on relative body surface areas. Other considerations include (1) the relationship of the dose at which immunotoxic effects were seen to doses causing other toxicities, (2) the doses at which pharmacological activity was observed, and (3) the reversibility of immunotoxic effects. Signs of immunotoxicity suggest that additional follow-up studies should be considered to better understand the underlying mechanism (unless the effect is tolerable for the intended use of the drug).

Effects on immune system parameters in nonclinical toxicology studies are often attributed to stress and are not considered toxicologically significant. In laboratory animals, certain environmental conditions, such as crowding, isolation, temperature, food or water deprivation, alteration of light-dark cycle, immobilization, handling, and drug administration procedures are known to have an effect on the immune system (Ader and Cohen 1993). Such stress-related changes are often reversible with repeated dosing and might not be dose-related. A comparison of observed effects with vehicle-treated controls might be useful to determine whether there are toxicological effects of the drug that are stress inducing.

The pharmacological effects of the drug should be considered (e.g., where adverse immune changes result indirectly from effects of the drug on the central nervous system or the hypothalamic-pituitary-adrenal axis). When adverse effects do not suggest a stress reaction or are not related to the

pharmacological properties of the drug, the possibility exists that the drug has a direct adverse effect on the immune system. Even when there are potential indirect mechanisms for alterations in immune parameters, the patterns should be carefully evaluated to determine whether additional immunotoxicity studies would be useful.

Results of nonclinical pharmacokinetic studies should also be evaluated. For example, if distribution studies indicate that the drug concentrates in reticuloendothelial tissues (usually macrophages) and no signs of immunotoxicity were apparent in the toxicology studies, conducting a study on the impact of this observation (such as determining potential adverse effects on macrophage function) should be considered.

IV. IMMUNOSUPPRESSION

The term *immunosuppression* refers to an unintended impairment of any immune component or function. Indicators of immunosuppression can be observed in standard nonclinical toxicology studies and include:

- Evidence of myelosuppression, such as pancytopenia, anemia, leukopenia, lymphopenia, thrombocytopenia, or other blood dyscrasias

- Alterations in histology, including thymic atrophy or hypocellularity of immune system tissues such as the spleen, lymph nodes, or bone marrow

- Increased incidence of infections

- Increased incidence of tumors

- Decreased serum immunoglobulin levels

It is important to differentiate between unintended (adverse) immunosuppressive effects and intended (pharmacodynamic) effects. For example, many antitumor drugs are toxic to rapidly dividing cells. Immunosuppression due to bone marrow toxicity would be considered an adverse effect during the treatment of a solid tumor, but not during treatment of a hematologic malignancy. For drugs intended to be used for prevention of transplant rejection (e.g., cyclosporine), immunosuppression is the intended pharmacodynamic effect.

A. Detection of Immunosuppression

All investigational new drugs should be evaluated for unintended immunosuppression. This can be accomplished in repeat-dose toxicology studies by examining clinical chemistry and hematology (including differential) values, gross pathology findings, immune system-related organ weights, and histopathological results in immune system-related tissues. Histopathology should

include examination of spleen, thymus, lymph nodes, and bone marrow. In addition, the lymphoid tissue that drains or contacts the site of drug administration (and therefore is exposed to the highest concentration of the drug) should be specifically examined. These sites are the gut-associated lymphoid tissues (GALT) for oral administration and the draining lymph nodes for intramuscular, intradermal, or subcutaneous administration. For intravenously administered drugs, the spleen can be considered the draining lymphoid tissue.

When changes are observed such as depletion or hyperplasia in lymph nodes or splenic white pulp, changes in cortical (T-cell) or medullar (B-cell) areas should be noted. Other indicators of immunosuppression in nonclinical toxicology studies include treatment-related infections and lymphoproliferative type tumors. Although decreases in serum immunoglobulin might be considered a relatively insensitive indicator of immunosuppression, this measurement is useful because it can be readily incorporated into the standard battery of clinical pathology tests.

Changes in blood cellular elements can suggest immunosuppression, but evaluation can be complex. Anemias and other blood dyscrasias can be associated with effects ranging from direct bone marrow toxicity to hemolysis caused by drug-induced anti-erythrocyte antibodies. Differentiating direct bone marrow toxicity or direct drug-mediated intravascular hemolysis from immune-mediated cytolysis in immunosuppression can be difficult. Direct bone marrow toxicity is usually determined by histopathology. Several in vitro methods can be used to determine the bone marrow cell targets of cytotoxicity (Deldar et al., 1995). Direct intravascular hemolysis is frequently accompanied by increases in white cell counts, increased spleen weight, hemosiderosis of various tissues, and reticulocytosis. Drug-mediated hemolysis can sometimes be confirmed by in vitro assay (incubating the drug with erythrocytes and determining release of hemoglobin). Detection of cell-bound antibodies can determine whether the immunosuppressive effect has an autoimmune or antidrug antibody component. This mechanism of immunosuppression, however, is rarely observed in standard nonclinical toxicology studies.

The timing of the onset of any dyscrasia should be carefully evaluated. Cell loss in circulation resulting from damage to marrow cells follows a time course that reflects the half-life of the cell type. For example, with damage to an early stem cell, granulocytopenia is likely to be observed first, followed by thrombocytopenia. Anemia will appear much later, reflecting the long lifetime of red blood cells. If the loss of a specific cell type is inconsistent with marrow damage, direct attack on mature cells might be indicated. Although follow-up studies are not generally essential to support the safety of a new drug, they may be useful in the risk/benefit analysis.

B. Immune Cell Phenotyping

If signs of immunosuppression are observed in nonclinical toxicology studies, follow-up studies to determine potential mechanisms are encouraged. Findings from such studies could suggest modifications to trial entry criteria or guide the management of adverse symptoms. For the purposes of drug development, the determination of drug effects on immune cell phenotypes may be useful. Consultation with FDA staff can be helpful during study design, but as a general

practice, cell surface phenotype determinations should be made on splenocytes obtained at necropsy and, when practical, on circulating white blood cells. These determinations should be conducted ideally as a part of one or more repeat-dose toxicology studies using flow cytometry or comparable methodology.

The choice of markers to be quantitated should be based on the intended use of the drug and toxicities observed in previous studies. These can include CD4 and CD8 for T-cells, surface immunoglobulin or other markers for B-cells, and an NK cell marker (such as CD16 or CD56). Immunohistochemical analysis has also been found useful. This technique can be particularly helpful in characterizing histological changes in immune cell tissues.

Although immune cell phenotype determination is not generally considered to be a test of drug effects on immune function, it may be useful for two reasons: (1) immune cell phenotype changes (as determined by flow cytometry) have demonstrated one of the best single correlations with host resistance against pathogens or tumors in studies conducted by the National Toxicology Program (NTP) (Luster et al., 1993); and (2) the method can be effectively used to monitor adverse effects in clinical trials (Selgrade et al., 1995). Both percentages and absolute cell counts can be determined by a single method (Cornacoff 1995). In addition, flow cytometry can be combined with tests of immune function for assessment of immunosuppressive potential (Luster et al., 1992a).

C. Immune Function Studies

When warranted by observations in nonclinical toxicology studies, additional studies to determine potential drug effects on immune function should be considered. The dose, duration, and route of administration in any immune function study should be consistent with the study in which an adverse effect was observed, and the assays should be based on the type of adverse effects observed. For example, if decreases in total lymphocytes or specific T-cells (e.g., CD4 cells) or increased infections were observed, a study to determine the effect of drug exposure on T-cell-dependent antibody response could be conducted. The anti-sheep red blood cell (SRBC) IgM antibody response assay (usually referred to as the *plaque assay*), which has been extensively evaluated by the NTP, could be used for this purpose (Luster et al., 1988, 1992b, 1993). This is normally conducted as a stand-alone assay. However, there is a version in which the assay is integrated into standard nonclinical toxicology studies. Animals in the study are immunized with an antigen (e.g., SRBC, tetanus toxoid) and antibody response is determined by enzyme-linked immunosorbent assay (ELISA) (Ladics et al., 1995). Although the ELISA variation is not a true test of immune function, it has demonstrated a high correlation with the plaque assay (Holsapple 1995; Temple et al., 1993, 1995).

In addition, when treatment-related infections are observed in nonclinical toxicology studies, the cause of infections should be determined. Infections originating from weakly pathogenic organisms could be an important indicator of unintended immunosuppression.

Depending on the effects, any assay of immune function for which there is a valid scientific rationale could be used in follow-up studies. Useful information for assessing effects of drugs on immune function has been obtained from assays of natural killer cell function, in vitro blastogenesis, cytotoxic T-cell function, specific cytokine production, delayed-type hypersensitivity response, and host resistance to experimental infections or implanted tumors (host resistance assays). In vitro assays using human peripheral mononuclear cells may provide additional information.

The importance of follow-up immune function studies for overall safety assessment depends on the intended use of the drug. If a drug is likely to be used in pregnant women (for example, to prevent perinatal transmission of an infectious disease such as HIV), immunotoxicology determinations in the ICH Stage C-F reproductive toxicology study should be considered. Ideally, the effect of maternal drug exposure on lymphoid system histopathology and hematology in the F_1 generation offspring should be included in the terminal assessments.

If a drug is intended for treatment of HIV infection or a related immune disease, immune function studies should be considered part of the safety assessment, even when no signs of immunotoxicity have been observed in the standard toxicology studies. Because of the presumed increased susceptibility to drug-associated immunotoxicity of patients with impaired immune function, extra nonclinical effort to detect immunotoxic effects is warranted.

V. ANTIGENICITY

Antigenicity is a complex problem in drug development and is one that depends on a number of factors, including molecular size, configuration, charge, accessibility of determinant groups, and digestibility. True antigens are digestible by antigen-presenting cells (APC). Although antigens are usually proteins, antigenic polymers such as polysaccharides, nucleic acids, and other macromolecules have been described. The species, route, and schedule of administration, metabolism of the drug, and amount of immunogen (e.g., high dose tolerance) can all affect antigenicity.

There are two major concerns associated with antigenicity in drug development: (1) the ability of antidrug immune responses to alter the biological activities of the drug (pharmacodynamics, toxicities, and/or pharmacokinetics), and (2) hypersensitivity responses (See section VI). Concerns about alterations in biological activities are most often associated with peptide, polymer, and protein drugs. Antidrug antibody responses can neutralize drug activity and alter drug clearance, plasma half-life, and tissue distribution.

Drugs can be grouped into two major classes with respect to their potential antigenicity: low molecular weight compounds ($\# 1{,}000\ M_r$) and peptides, polymers, or proteins with molecular weights $\$ 10{,}000\ M_r$. Examples of low molecular weight drugs that can be antigenic include penicillin and sulfonamides. Their antigenicity depends on covalent binding of parent drugs or metabolites to proteins to form hapten-

protein conjugates. Larger peptides, polymers, or protein drugs with molecular weights $ 10,000 M_r are usually antigenic. Smaller peptides or proteins in the 5,000 to 10,000 M_r range also may be antigenic, although immune responses to these drugs may be fairly weak. Antigenicity is unpredictable for compounds in the 1,000 to 5,000 M_r range.

Under certain circumstances, attempts should be made to determine the antigenic potential of large molecular weight drugs. Although demonstrating antigenicity in an animal model does not necessarily predict antigenic potential in humans, there are other reasons why it might be important to monitor antidrug immune responses. For example, antidrug immune responses could complicate the determination of safe starting doses for clinical trials or lead to an incorrect interpretation of pathological findings of direct target organ toxicity. Pharmacokinetic parameters may be altered so that observed effects may not indicate the true toxic potential of the drug.

Assays to identify antidrug immune responses (such as ELISA and/or lymphocyte blastogenesis assays) should be considered part of nonclinical safety assessment, because peptide, polymer, and protein drugs and classes are known to be potentially haptenic (e.g., penicillins). Such assays can be useful not only for interpreting animal studies, but also for assessing the extent of an antidrug immune response in clinical trials. Immunoassays for specific cell-mediated immunity should also be considered.

VI. HYPERSENSITIVITY (DRUG ALLERGY)

Hypersensitivity refers to antigen-specific immunological reactions that have adverse effects. The classification system discussed below includes four classes of hypersensitivity responses (Coombs and Gell 1975):

- Type I, IgE mediated — immediate-type hypersensitivity
- Type II, IgG or IgM mediated — antibody-mediated cytotoxic reactions
- Type III, IgG mediated — immune complex reactions
- Type IV, T lymphocyte mediated — delayed-type hypersensitivity response

Small molecular weight drugs are allergenic if they bind directly to proteins, either as the parent drug or via metabolites. Drugs that are antigenic may or may not be allergenic. The type of hypersensitivity reaction depends on many factors, such as degree of antigenicity of the drug, the type of antibody produced (IgE, IgG, IgM), the route of administration (oral, intramuscular, intravenous, topical), the dosing regimen (acute versus chronic), and the pharmacokinetics and metabolism of the drug. Assays for these types of reactions are discussed in the following sections.

A. Type I

Type I hypersensitivity reactions are mediated by IgE in humans. Assays should detect the ability of a drug to elicit production of this type of antibody (or, in the case of guinea pigs, IgG_1,

which mediates immediate-type responses in this species). Two tests for anaphylactic reactions are commonly used: passive cutaneous anaphylaxis (PCA) and active systemic anaphylaxis (ASA). Neither the PCA nor ASA is generally considered an essential test for safety assessment of drugs. They also are not useful for exploring mechanisms of hypersensitivity. If a small molecular weight drug induces IgE antibody production in the PCA assay, the drug might have sensitizing (allergenic) potential. However, a negative result in the PCA assay does not necessarily indicate that a small molecular weight drug lacks sensitizing potential, especially when biotransformation would be necessary for production of potential haptens.

The ASA assay can determine whether a test drug can induce anaphylaxis in an animal following immunization with the drug. As with the PCA assay, this method detects the ability of proteins and protein-reactive compounds to produce anaphylaxis. Like the PCA assay, however, the ASA assay might not be appropriate for determining the sensitizing potential of nonreactive small molecular weight drugs (where metabolism is necessary for production of hapten), and negative findings should not be interpreted to indicate that an experimental drug cannot produce anaphylactic reactions. The usefulness of this assay for the safety assessment of drugs is thus considered limited.

Methods have been developed that can detect IgE production in mice following dermal exposure to a test substance. For example, the mouse IgE test (MIGET) has been used to detect respiratory sensitizers (Hilton et al., 1995). Serum cytokine patterns following topical exposure in mice have also been used to detect respiratory sensitizers (Dearman et al., 1995, 1996). The MIGET and determination of cytokine patterns, conducted in tandem with the murine local lymph node assay (LLNA, discussed under Type IV reactions), might be useful in detecting respiratory allergens (generally associated with Type I immunopathy) (Kimber et al., 1996). However, these assays have not been demonstrated to model anaphylaxis, especially when biotransformation appears to be necessary for production of hapten(s).

Adaptations of guinea pig assays to detect contact sensitizers (Type IV hypersensitivity reactions) can evaluate the potential of drug products administered by inhalation to induce Type I hypersensitivity responses in the respiratory tract (DeGeorge et al., 1997). The suggested tests involve dermal or inhalation induction followed by inhalation challenge, using plethysmography and other experimental endpoints to determine sensitization (Karol 1995). Drugs intended for inhalation should be tested for their sensitizing potential.

B. Type II & III

Type II and III immunopathies tend to occur simultaneously and are commonly associated with systemic or specific organ hypersensitivity reactions. Type II and III immunopathies are the result of IgG and/or IgM antibody responses to drugs or drug metabolites. The associated pathologies are due to antibody-dependent cellular cytotoxicity (ADCC) and/or complement mediated lysis of somatic cells (Type II) or immune complex formation, deposition, and complement activation with local tissue destruction (Type III). Type II and III immunopathies

include anemia, leukopenia, thrombocytopenia, pneumonitis, vasculitis, lupus-like reactions, or glomerulonephritis, and are often indistinguishable from autoimmune reactions. Type II and III immunopathies appear to be only rarely modeled in animals and signs of these immunopathies are most commonly indicative of direct, nonimmune-mediated drug toxicity.

Although there are examples of drugs that are associated with Type II and III hypersensitivity reactions, there are no standard nonclinical methods for predicting these effects. There are instances, however, when follow-up studies should be considered to determine if immune mechanisms are involved in these pathologies. In the case of anemia, a positive direct Coombs test could indicate an immune-mediated hemolytic anemia. In the case of specific tissue damage, such as vasculitis, immunohistochemical demonstration of antibody or complement in the affected tissue could suggest immunopathy. Demonstration of immune complex formation with peptide and protein drugs in animal studies does not directly predict the potential for immune complex disease in humans. Such findings, however, should be carefully considered, especially when immune complex deposition leads to pathological effects. The consequences of immune complex formation may also include neutralization of drug activity and changes in pharmacokinetics.

In certain instances, specialized biomarker assays can be useful for understanding mechanisms when a drug belongs to a chemical class known to be associated with specific immunopathies. For example, the inhalation anesthetic halothane is known to cause severe liver damage in rare instances, and this effect appears to have an immunologic basis (Pohl et al., 1988). Antibodies reactive with liver metabolites of halothane are associated with halothane hepatitis (Hubbard et al., 1988; Kenna et al., 1984) and these metabolites have been identified as trifluoroacetylated proteins (Pohl et al., 1989). Compounds that are chemically related to halothane can be administered to guinea pigs to determine the formation of hepatic trifluoroacetylated proteins (Clarke et al., 1995). This biomarker might be useful for indirectly assessing the sensitizing potential of chemicals related to halothane (Furst et al., 1996).

C. Type IV

Type IV immunopathies are T-cell mediated and most commonly occur as delayed-type hypersensitivity skin reactions (contact dermatitis). When a drug is intended for topical administration, the sensitizing potential of the drug should be determined using an appropriate assay as part of nonclinical safety evaluation. The classic nonclinical studies use sensitization (induction) and challenge (elicitation) and are typically conducted in guinea pigs. Although numerous assays have been developed, the most common methods for evaluating the dermal sensitizing potential of drugs have been the Buehler test and the guinea pig maximization test (Botham et al., 1991). These methods are considered very reliable and have demonstrated a high correlation with known human skin sensitizers (Kligman and Basketter 1995). These methods, along with the split adjuvant technique and the Draize test, are currently accepted by the FDA for determining the sensitizing potential of drugs intended for topical use. Other methods (such as the optimization assay) have also been used for the nonclinical evaluation of

topical drugs and have been accepted by FDA. Techniques using mice, rather than guinea pigs, have also been developed. The mouse ear-swelling test (MEST) (Gad et al., 1986, 1987) uses an induction and challenge pattern similar to the traditional guinea pig tests.

Experimental techniques that detect the induction phase of delayed-type hypersensitivity reactions may be useful in drug development. One technique in particular, the murine LLNA, has been the subject of several studies with known contact sensitizers (Basketter et al., 1991; Kimber et al., 1991; 1995; Loveless et al., 1996; Scholes et al., 1992). With some limitations (e.g., whether test compound is soluble in an appropriate vehicle), the LLNA can be used as a stand-alone alternative to standard guinea pig tests for the detection of contact sensitizers. The test is designed to detect in situ lymphoproliferation. Studies have indicated that the LLNA results correlate well with traditional guinea pig tests for industrial chemicals (Basketter and Scholes, 1992; Basketter et al., 1993; Edwards et al., 1994; Kimber et al., 1990). Studies with pharmaceutical compounds have demonstrated similar reliability (Kimber et al., 1998; Robinson and Cruze 1996). The LLNA may have advantages over guinea pig tests. The results are quantitative rather than essentially subjective; Freund's adjuvant is not used; and colored products can be accurately assayed. In addition, adaptations of the LLNA have been reported in which lymph node cell phenotypes determined by flow cytometry have been shown to distinguish irritants from allergens. LLNA results can support the safety of proposed clinical trials with topical drug products.

Photoallergy is a special case of Type IV hypersensitivity in which photoactivation of a drug results in a covalent-binding metabolite (hapten), which then acts as a sensitizer. Animal models may be useful for evaluating photoallergenic potential (Gerberick et al., 1989; Scholes et al., 1991), but the predictive value of these models for human effects is uncertain. For this reason, nonclinical testing for photoallergenic potential is not routinely expected by FDA for topical drugs.

Other determinations could be valuable in assessing the sensitizing potential of experimental drugs. Although covalent binding to proteins in itself should not be considered a predictor of allergenic potential, in certain situations it could be important. Thus, if a drug belongs to a class known to produce hypersensitivity reactions through covalent binding (e.g., β-lactams, sulfonamides), demonstration of in vivo covalent binding to proteins could be taken as a biomarker of sensitization potential (Dewdney and Edwards 1992).

D. Pseudoallergic (Anaphylactoid) Reactions

A pseudoallergic reaction can result from activation of inflammatory or anaphylactic mechanisms independent of antigen-specific immune responses. Pseudoallergy is known to have several causes, including but not limited to direct histamine release and complement activation. This reaction is likely to be dose-related.

If signs of anaphylaxis are observed in animal studies (e.g., cardiopulmonary distress), follow-up studies should be considered. Anaphylactoid reaction can be differentiated from true IgE mediated anaphylaxis by various methods, including in vitro testing. Biochemical markers of the anaphylactoid reaction can be observed following direct addition of the drug to human serum (to test for complement activation) or to cells to measure histamine release. Careful evaluation of these reactions has resulted in valuable information on biochemical markers used in clinical trials.

VII. AUTOIMMUNITY

Autoimmunity refers to a pathological process in which the immune system responds to self-antigens. Autoimmune targets include functional membranes (such as the renal glomerulus), protective membranes (such as myelin), or receptors (such as thyroid stimulating hormone or acetylcholine receptors). Glomerulonephritis, lupus-like syndrome, hemolytic anemia, and vasculitis are among the most common pathologies thought to have an autoimmune basis. The effectors of autoimmunity can include antibodies or T-cells specific for self-antigens. The consequences of autoimmunity include direct tissue damage, immune complex deposition with complement activation, or stimulation of target function. Type II and III hypersensitivity reactions often have autoimmune components, and drug-associated autoimmunity can originate as a drug-specific hypersensitivity reaction. Immune stimulation due to specific immune reactions (stimulatory hypersensitivity) may be considered a type of autoimmunity.

There are no standard methods for determining the potential of experimental drugs to produce autoimmune reactions. The popliteal lymph node assay (PLNA) and various adaptations of it have been proposed to test for autoimmunity induction by drugs (Descotes and Verdier 1995). This method may have promise, but no extensive evaluation has been reported that would support any recommendation for drug development.

Other methods that have been proposed include assay for markers of T-cell activation (e.g., soluble IL-2 receptor) and effects of a drug on markers of T_H2 cell induction in the Brown Norway rat. Although there are instances in which autoantibody formation has been demonstrated in nonclinical toxicology studies, these observations have not been shown to predict the ability of experimental drugs to induce autoimmune disease in humans.

VIII. ADVERSE IMMUNOSTIMULATION

Adverse immunostimulation refers to any antigen-nonspecific, inappropriate, or unintended activation of some component of the immune system. Chronic inflammation can be considered to result from adverse immunostimulation, although it is more likely to be associated with products such as implanted medical devices and vaccine adjuvants than with drug products.

Unintended nonspecific immunostimulation appears to be a relatively unusual adverse effect. In some respects, this class of immunotoxicity overlaps with pseudoallergy and, in fact, the distinction is subtle.

Compounds with this type of activity are often proposed for use as immune stimulants (e.g., adjuvants) and in this instance adverse immunostimulation would be considered exaggerated pharmacodynamic activity.

The clinical manifestations of such activity pose a diagnostic challenge due to the variety of cells and tissues that could be affected by overstimulation. A relatively common manifestation of immunostimulation is leukocytic infiltration of tissues. Adverse immunostimulation can be difficult to identify because the observed effect may not be in an immune system component. For example, the limiting toxicity of the immunostimulant interleukin-2 at high doses is diffuse capillary leakage. There are no nonclinical tests specifically designed to detect these effects.

IX. SAFETY CONSIDERATIONS

Specific nonclinical immunotoxicology studies for assessing safety or for exploring mechanisms of immunotoxicity are summarized in Attachments 1 and 2. As the flowchart in Attachment 1 indicates, additional immunotoxicology studies to complement the standard repeat-dose toxicology studies are expected when the drug is administered by inhalational or topical routes. For drugs administered by these routes, the sensitizing potential of the drug should be screened using an appropriate test such as the guinea pig maximization test (GPMT), Buehler assay (BA), local lymph node assay (LLNA), or mouse IgE test (MIGET).

Another concern in safety assessment is whether the drug is likely to be susceptible to antidrug immune responses. Drug antigenicity can alter activity, toxicity, or pharmacokinetics, or cause hypersensitivity reactions — all of which can generate safety concerns. For large molecular weight drugs or drugs from structural classes known to be haptenic, diligent attempts should be made to detect antidrug immune responses.

If a drug is expected to be used in pregnant women, incorporation of immunotoxicology in the ICH Stage C-F reproductive toxicology study should be considered. Ideally, the effect of maternal drug exposure on lymphoid system histopathology and hematology in the F_1 generation offspring should be included in the terminal examination.

After determining whether specific testing is warranted based on the route, the potential for eliciting antidrug immune responses, or intended use in pregnant women, the repeat-dose toxicology studies conducted to support the safety of a drug in clinical trials should be carefully examined. If evidence of drug immunotoxicity is found, Attachment 2 suggests follow-up studies for immunotoxicity that should be considered, depending on the finding. In addition to immunotoxicity findings in the repeat-dose studies, tissue distribution studies should be considered. Drug accumulation or retention in reticuloendothelial tissues may increase the potential for immunotoxicity.

For drugs with these distribution properties but with no detectable signs of immunotoxicity, additional studies should be considered to determine whether immune function is indeed compromised. When no

accumulation or retention is evident in these tissues, the final consideration is whether the drug is intended for the treatment of HIV infection or a related immune disease. If this is the case, immune function tests should be conducted to assess immune function, even when no immunotoxicity was detected in the standard toxicology studies. This immune function testing will provide additional safety assurance for subjects in whom immunotoxicity could have serious consequences. If the drug will not be used to treat diseases with seriously impaired immune function, further immunotoxicity testing is not generally necessary unless warranted by specific clinical observations.

If signs of unintended immunotoxicity are observed in nonclinical toxicology studies, appropriate follow-up studies should be considered (Attachment 2). These follow-up studies are usually not essential for assessing drug safety, but can provide information useful for the risk/benefit assessment. For further evaluation of immunosuppressive effects, two assays in particular should be considered: (1) immune cell phenotyping (by flow cytometry) and (2) the anti-sheep red blood cell plaque assay. An in vitro hemolysis assay can help ascertain whether blood dyscrasias are due to immune-mediated cytolysis or direct drug effects.

When hypersensitivity reactions are observed in toxicology studies, additional immunotoxicity testing might be useful for clarifying the immune system's role. For example, when anemia is present, a Coombs test could indicate whether immune-mediated hemolytic anemia is the cause. Likewise, to explore specific tissue damage, such as vasculitis, demonstration of immune complex deposition in the affected tissue would indicate an immunopathologic mechanism. Follow-up studies can also differentiate anaphylactoid reactions from true IgE mediated anaphylaxis. For example, drug induced histamine from cells or complement activation following addition of drug to serum would indicate the former.

Drug-induced autoimmunity suspected in toxicology studies is difficult to confirm with current methods. Nonetheless, the popliteal lymph node assay and specific biomarker assays might provide insight into potential autoimmune mechanisms.

The final indication of whether to undertake additional immunotoxicity testing is tumorigenicity. If chronic toxicology studies or rodent bioassays indicate carcinogenic potential, the contribution of unintended immunosuppression to the findings should be evaluated. If immunosuppression is suspected from other direct findings in the study or from class effects, follow-up studies should be considered. Tumor host resistance models are appropriate for determining carcinogenic immunosuppressive potential.

Immunotoxicity does not appear to be a common finding with investigational drugs. If a potentially valuable therapeutic agent has significant immunosuppressive activity in nonclinical toxicology studies, this would need careful attention in clinical trials. For instance, cancer therapeutic agents that are potent myelotoxins can be used if appropriate prophylactic measures are used to avoid infections. Certain combinations of drugs may be contraindicated when both drugs are human immunosuppressants. Relevant information on potential immunosuppressive activity should be included in the product labeling.

X. SUMMARY

The immune system consists of a diverse and complex set of cells and organs that have complicated interactions with each other and with other physiological systems. These complexities make the detection and evaluation of drug-induced immunotoxicity in animal models difficult. Nonetheless, regulatory considerations for immunotoxicologic effects discovered during the development of a drug are really no different than for other adverse effects. Immunotoxicologic findings could suggest additional follow-up studies to investigate the nature and mechanism of the immunotoxic effects. Any further testing should depend on the intended use of the drug, whether immunotoxicity is an expected or tolerable side effect, and whether results from additional testing would alter the clinical development plan, including potential risk/benefit considerations. Modifications in clinical trials could be indicated by immunotoxicity findings (e.g., certain immune parameters might be monitored). Immunotoxicity findings could be included in the investigator's brochure or in the product label. Finally, although immunotoxicity findings could indicate that a drug is unsafe for some types of clinical investigations or certain indications, this appears to be rare.

For the safety assessment of investigational new drugs, specific immunotoxicity testing should be conducted (in addition to the standard toxicology studies in two species) when drugs are to be administered by the inhalational or topical routes. For these drugs, tests predictive of contact sensitization should be conducted. The murine LLNA should be considered a validated stand-alone test for this purpose. Specific immunotoxicity studies should also be considered for safety assessment when (1) the drug has the potential to elicit an antidrug immune response, (2) use during pregnancy is likely, or (3) there is an absence of immunotoxicity findings in the toxicology studies, but significant accumulation or retention of the drug in immune system tissues, or the drug will be used to treat an immune-deficiency disease such as HIV. In most other instances, specific immunotoxicity studies are generally not needed to support initial clinical trials or continued development. If immunosuppression is observed in relevant toxicology studies, immune cell phenotyping and the anti-SRBC plaque assay (or immunoassay variants such as ELISA) are recommended for follow-up.

Immunotoxicology is a rapidly advancing field and new methods are constantly being evaluated. It is anticipated that new methods will become available for endpoints for which no generally accepted predictive tests currently exist, especially concerning such adverse effects as systemic hypersensitivity, autoimmunity, and photoallergy. Sponsors are encouraged to contact the appropriate review division when signs of immunotoxicity in toxicology studies or clinical trials suggest follow-up studies.

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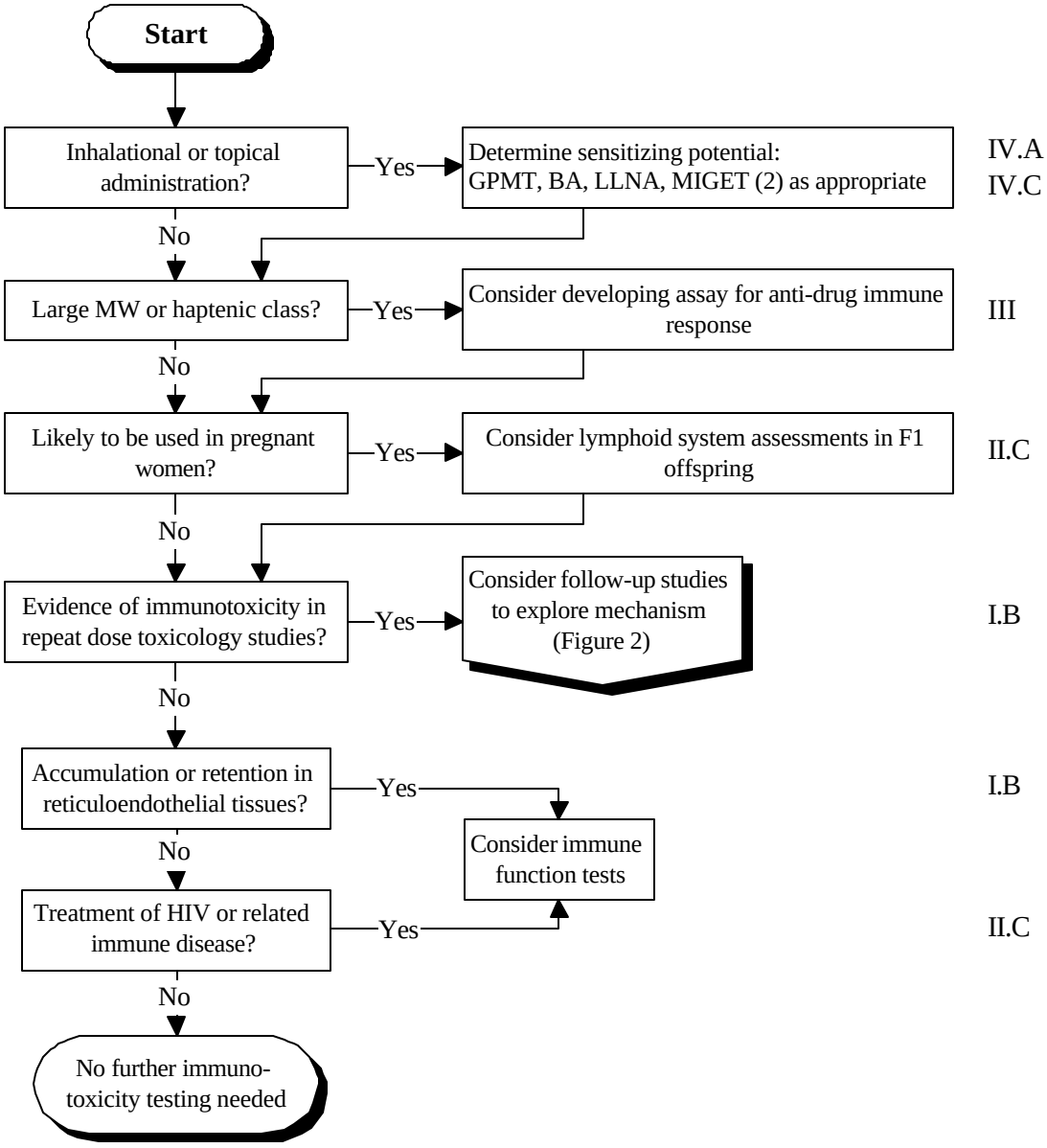
ATTACHMENT 1

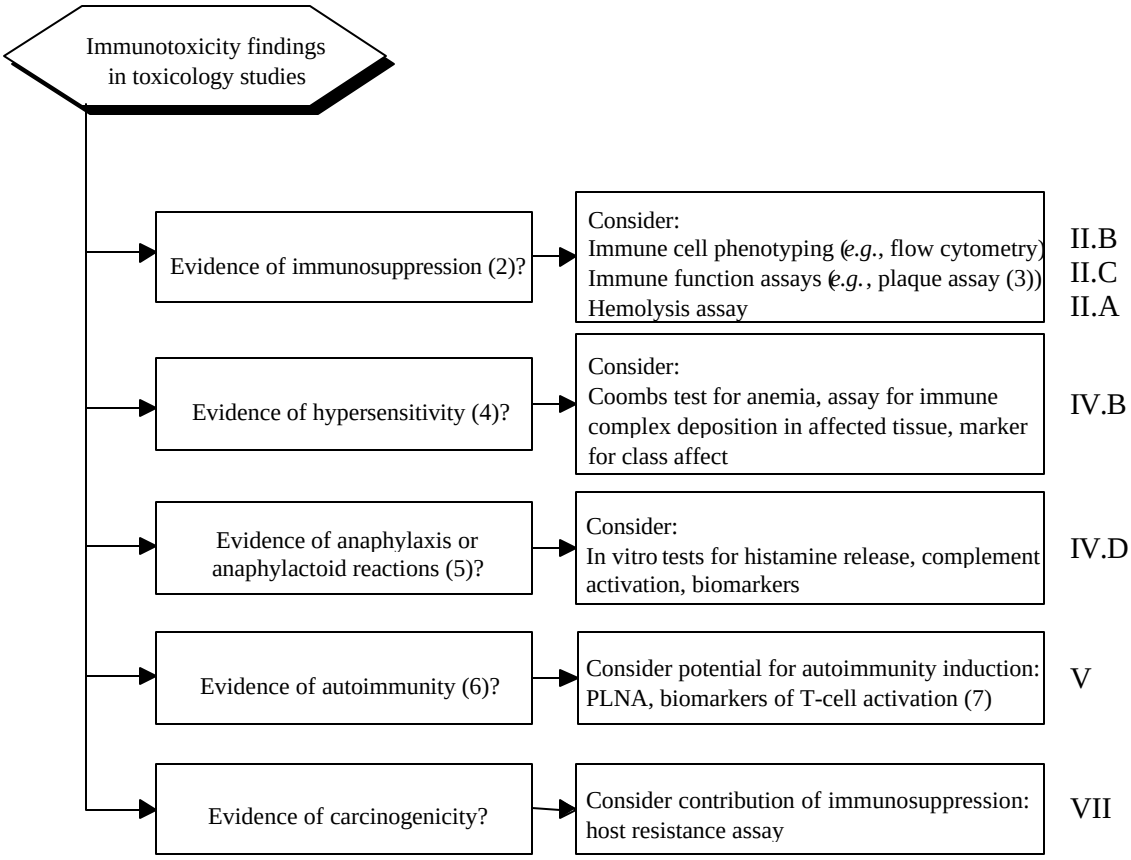
FLOWCHART FOR DETERMINING WHEN TO CONDUCT SPECIFIC IMMUNOTOXICITY TESTING



1. Annotations in right margin indicate location of text describing specific advice
2. GPMT- guinea pig maximization test; BA- Buehler assay (Buehler patch test); LLNA- local lymph node assay; MIGET- mouse IgE test (there is only a relatively small database available for assessing the usefulness of the MIGET for drug regulatory purposes)

ATTACHMENT 2

FOLLOW-UP STUDIES TO CONSIDER FOR EXPLORING MECHANISMS OF IMMUNOTOXICITY



1. Annotations in right margin indicate location of text describing specific advice.
2. Examples include myelosuppression, histopathology in immune associated tissues, increased infection, Tumors, decreased serum Ig, phenotypic changes in immune cells.
3. Other acceptable assays include drug effect on NK cell function in vitro blastogenesis, cytotoxic T cell function, cytokine production, delayed-type hypersensitivity, host resistance to infections or implanted tumors.
4. Examples include anemia, leukopenia, thrombocytopenia, pneumonitis, vasculitis, lupus-like reactions, Glomerulonephritis.
5. Examples include cardiopulmonary distress, rashes, flushed skin, swelling of face or limbs.
6. Examples include vasculitis, lupus-like reactions, glomerulonephritis, hemolytic anemia.
7. There are no established assays that reliably assess potential for autoimmunity and acute systemic hypersensitivity. The popliteal lymph node assay (PLNA) has only a relatively small database available for assessing its usefulness for drug regulatory purposes.